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(54) Title: PEPTIDES INVOLVED IN THE PATHOGENESIS OF HIV INFECTION

(57) Abstract

Peptides involved in the pathogenesis of human immunodeficiency virus ("HIV"). More particularly, this invention relates to peptides from the env region of the HIV genome and the use of such peptides in methods and compositions for preventing, treating, or detecting acquired immune deficiency syndrome ("AIDS") infection.

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PEPTIDES INVOLVED IN THE  
PATHOGENESIS OF HIV INFECTION

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TECHNICAL FIELD OF INVENTION

This invention relates to peptides involved  
10 in the pathogenesis of human immunodeficiency virus  
("HIV"). More particularly, this invention relates  
to peptides from the env region of the HIV genome  
and the use of such peptides in methods and composi-  
tions for preventing, treating, or detecting acquired  
15 immune deficiency syndrome ("AIDS") infection.

BACKGROUND ART

Acquired immune deficiency syndrome ("AIDS")  
is a disease characterized by severe or, typically,  
complete immunosuppression and attendant host sus-  
ceptibility to a wide range of opportunistic infec-  
tions and malignancies. AIDS' complete clinical  
manifestation is usually preceded by AIDS related  
complex ("ARCS"), a syndrome accompanied by symptoms  
such as lymphadenopathy, fever and weight loss.  
25 The human immunodeficiency virus ("HIV")  
retrovirus is thought to be the etiological agent

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responsible for AIDS infection and the ARCS syndrome [M. G. Sarngadharan et al., "Detection, Isolation, and Continuous Production of Cytopathic Retroviruses (HTLV-III) From Patients With AIDS and Pre-AIDS", 5 Science, 224, pp. 497-508 (1984)].\* Between 85 and 100% of the AIDS/ARCS patient population test sero-positive for HIV [G. N. Shaw et al., "Molecular Characterization of Human T-Cell Leukemia (Lymphotropic) Virus Type III In The Acquired Immune Deficiency 10 Syndrome", Science, 226, pp. 1165-70 (1984)].

Upon infection of a host, the primary targets of the HIV virus are T-4 lymphocytes, also known as helper or inducer cells. T-4 lymphocytes interact with other specialized cell types of the 15 immune system to confer immunity to or defense against infection. More specifically, T-4 lymphocytes stimulate production of growth factors which are critical to the functioning of the immune system. For example, they act to stimulate B cells, the descendants of 20 hemopoietic stem cells, which promote the production of defensive antibodies. They also activate macrophages ("killer cells") to attack infected or otherwise abnormal host cells, and induce monocytes ("scavenger cells") to encompass and destroy invading 25 microbes. Accordingly, when T-4 lymphocytes are rendered non-functional by HIV infection, this complex immune defense system is destroyed and the host becomes susceptible to a wide range of opportunistic infections. In addition to T-4 lymphocytes, the HIV 30 virus has also been shown to infect central nervous

\* In this application, human immunodeficiency virus ("HIV"), the generic term adopted by the human retrovirus subcommittee of the International Committee on Taxonomy of Viruses to refer to independent isolates from AIDS patients, including human T-cell lymphotropic virus type III ("HTLV-III"), lymphadenopathy-associated virus ("LAV") and AIDS-associated retrovirus ("ARV") will be used.

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system cells, macrophages and B lymphocytes [J. M. Ismach, "AIDS: Can The Nation Cope", Medical World News (August 25, 1985)].

5 The genome of retroviruses such as HIV contains three regions encoding structural proteins. The gag region encodes the core proteins of the virion. The pol region encodes the virion RNA-dependent DNA polymerase (reverse transcriptase). The 10 env region encodes the major glycoprotein found in the membrane envelope of the virus and in the cytoplasmic membrane of infected cells. The capacity of the virus to attach to target cell receptors and to cause fusion of cell membranes are two HIV virus properties controlled by the env gene. These 15 properties are believed to play a fundamental role in the pathogenesis of the virus.

HIV env proteins arise from a precursor polypeptide that, in mature form, is cleaved into a large heavily glycosylated exterior membrane protein 20 of about 481 amino acids -- gp120 -- and a smaller transmembrane protein of about 345 amino acids which may be glycosylated -- gp41 [L. Ratner et al., "Complete Nucleotide Sequence Of The Aids Virus, HTLV-III", Nature, 313, pp. 277-84 (1985)].

25 In the absence to date of effective treatments for AIDS, many efforts have centered on prevention of the disease. Such preventative measures include HIV antibody screening of all blood, organ and semen donors and education of AIDS high-risk 30 groups regarding transmission of the disease.

Experimental or early-stage clinical treatment of AIDS and ARCS conditions have included the administration of antiviral drugs, such as HPA-23, phosphonoformate, suramin and ansamycin, which 35 apparently interfere with replication of the virus by inhibiting its reverse transcriptase. Administration of some of these drugs in effective amounts

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has, however, been accompanied by undesirable and debilitating side effects. Other proposed methods for treating AIDS have focused the administration of alpha interferon or the application of hybridoma 5 technology. Most of these treatment strategies are expected to require the co-administration of immuno-modulators, such as interleukin-2. However, while some of these treatments are promising, none has been shown to be truly effective.

10 Recent studies have also demonstrated that HIV is experiencing genetic drift in humans. At least two classes of the virus have now been identified in AIDS patients in the United States. Furthermore, patients having high levels of HIV neutralizing 15 antibodies suffer more serious forms of the disease than those patients with poor neutralizing capabilities [Dr. William Haseltine, speech at Memorial Sloan-Kettering Cancer Center, October 9, 1985]. These recent observations suggest serious obstacles 20 to the development of an effective vaccine or monoclonal antibody-directed therapeutic method against HIV AIDS infections.

Accordingly, despite these developments to date, the need exists for the development of effective 25 agents for the prevention, treatment and diagnosis of HIV and AIDS-related infections.

#### DISCLOSURE OF THE INVENTION

The present invention solves the problems referred to above by providing peptides involved in 30 the pathogenesis of the HIV virus. According to one embodiment, the peptides of this invention are selected from the group consisting of peptides characterized by an amino acid sequence derived substantially from the region between about amino acid 35 600 and amino acid 750 of the HIV env gene. This region is believed to have an important role in

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virus-mediated pathogenic events. More preferably, the peptides of this invention consist substantially of the following amino acid sequences of the HIV env gene -- peptide 1: amino acids 616-632; peptide 2: amino acids 667-680; peptide 3: amino acids 627-639\*; peptide 4: amino acids 728-751 and peptide 64: amino acids 627-639. This invention also includes the D-retro form of each of the above-identified peptides -- those produced by synthesis with D amino acids in the opposite orientation, beginning with the carboxy terminal amino acid of the L form.

Other embodiments of this invention include peptide 5, which consists substantially of amino acids 426-450 of the HIV env gene, peptide 6, which 15 consists substantially of amino acids 496-519 of the HIV env gene, peptide 31, which consists substantially of amino acids 148-165 of the HIV env gene and peptide 78, which consists substantially of peptides 298-314 of the HIV env gene. This 20 invention also includes the D-retro form of each the above-identified peptides. These peptides produce antisera which, in conventional assays, bind to the HIV virus, inhibit syncytium formation or neutralize the virus. In addition, the peptides themselves may 25 be capable of inhibiting HIV-directed syncytium formation or neutralizing HIV in conventional assays. Such peptides, therefore, are useful in compositions

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30 \* Due to an inadvertent mislabelling of the peptide sample assayed, the 873,621 application referred to peptide 3 as amino acids 702-715 of the HIV env gene. Subsequent amino acid sequencing of the peptide sample demonstrated that the peptide labelled "peptide 3" 35 in fact had the sequence: amino acids 627-639 of the HIV env gene. As employed in the present application, therefore, "peptide 3" refers to a peptide having the sequence of amino acids 627-639 of the HIV env gene. This is the same sequence as peptide 64. Thus, peptides 3 and 64 are identical. The two numbers are used to distinguish the preparations of the 40 two peptides.

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and methods for preventing, treating and detecting AIDS infection.

- The peptides of this invention comprise functional regions of the HIV env protein involved in virus-mediated events, such as adsorption to normal cells and syncytium formation, which contribute to the pathogenesis of the disease. The functional regions encompassed by these peptides also correspond to immunogenic determinants of the HIV env gene which are highly conserved. Accordingly, these peptides comprise segments of HIV env protein which are highly immunogenic and are involved in virus pathogenesis over the range of genetic variants of the HIV virus.
- 15 The peptides of this invention may be advantageously used in vaccines or therapeutic compositions which elicit antibodies reactive with the native env protein of the HIV virus or which interfere with the virus by neutralization or inhibition 20 of syncytium formation. Furthermore, these peptides are easily modified in composition and conformation to improve the specific activity of those peptides against the HIV virus. In addition, these peptides may be used as diagnostic agents for detecting HIV 25 infections.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the amino acid sequences of each of peptides 1-6, 31, 64 and 78 of this invention, as well as that of the region between amino acid 600 and amino acid 750 of the HIV env gene. In this figure, the amino acids are represented by single letter codes as follows:

35 Phe: F    Leu: L    Ile: I    Met: M  
Val: V    Ser: S    Pro: P    Thr: T  
Ala: A    Tyr: Y    His: H    Gln: Q  
Asn: N    Lys: K    Asp: D    Glu: E  
Cys: C    Trp: W    Arg: R    Gly: G

DETAILED DESCRIPTION OF THE INVENTION

Referring now to Figure 1, we prepared various peptides corresponding to segments of the env gene of the HIV genome and tested them in 5 several conventional assays to demonstrate that they display activities reflecting their involvement in virus-mediated pathogenic events.

It should be understood that the present invention is not limited to the illustrative peptides depicted in Figure 1. Instead, a peptide falling within the scope of this invention may extend outside of or comprise less than the region between amino acid 600 and amino acid 750 of the HIV env gene, as long as a substantial part of that peptide is characterized by an amino acid sequence from 10 that region, or segments or combinations thereof, and that peptide demonstrates the desired immunological or biological activity against HIV. In addition, peptides according to this invention include 15 those having amino acid sequences which are longer or shorter in length than those of peptides 1-4 and 64 or which comprise segments or combinations thereof, as long as such peptides consist substantially of the region between amino acids 600-750 of 20 the HIV env gene and demonstrate the desired immunological or biological activity. Furthermore, peptides according to this invention include those 25 characterized by a sequence of amino acids which is longer or shorter than that of any one of peptide 5, peptide 6, peptide 31 or peptide 78, or which comprise segments of each of those peptides and which display 30 immunological or biological activity against HIV.

Accordingly, it should be understood that the specific selection of any one peptide within the 35 peptides of this invention is not critical. Such a selection may be carried out by taking a number of peptides and testing them for their immunological

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and biological activity against HIV as described herein.

The peptides according to this invention may be prepared by conventional synthesis using any of the known peptide synthesis methods, including synthesis on a solid support. The peptides of the invention may also be prepared in appropriate hosts transformed with DNA sequences that code for the desired peptide. For example, a peptide of this invention may be prepared by the fermentation of appropriate hosts that have been transformed with and which express a DNA sequence encoding that peptide. Alternatively, DNA sequences coding for several of the peptides of this invention may be linked together and those sequences may then be used to transform appropriate hosts to permit the expression of peptides involved in the pathogenesis of HIV infection. A combination of such methods may also be employed. In a preferred embodiment of this invention, chemical synthesis alone is employed. By means of that method, the peptides of this invention are additionally advantaged because they are easily purified and are non-biological in origin.

The peptides of this invention are preferably coupled to one or more carrier proteins, such as keyhole limpet hemocyanin ("KLH") before use in the compositions and methods described herein. The peptides are coupled to the carrier protein in various conventional ways, such as those described by M. Reichlin, "Use Of Glutaraldehyde As A Coupling Agent For Proteins And Peptides", Methods In Enzymology, 70, pp. 159-65 (1980).

After preparing the peptide and coupling it to the carrier protein, if desired, the antigen is employed in the methods and compositions of this invention in a conventional manner. For example, the peptide or coupled peptide, alone or in combi-

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ation with other peptides of this invention, is usually mixed with one or a combination of well-recognized adjuvants and additives, preferably by first dissolving the peptide, for example, in PBS 5 with 0.1% SDS. In other embodiments of this invention, the peptides may be linked to hydrophobic groups to build the adjuvant into the composition. Of course, it should be understood that other well-known methods of preparing therapeutic compositions 10 may be employed using the peptides of this invention.

The above-prepared compositions are then employed in a conventional manner for the treatment of HIV infections. Such methods of treatment and their dosage levels and requirements are well- 15 recognized in the art and may be chosen by those of skill in the art from available methods and techniques. For example, the peptides of this invention may be combined with a pharmaceutically acceptable adjuvant for administration to an HIV-infected 20 patient in a pharmaceutically acceptable manner and in an amount effective to lessen the severity of the HIV infection. The dosage and treatment regimens will depend upon factors such as the patient's health 25 status, the severity and course of infection and the judgment of the treating physician.

Alternatively, the peptides of this invention are useful in vaccines and methods for protecting humans against HIV infection for at least some period of time. The peptides may be employed in 30 these vaccines and methods either alone or together with other peptides of this invention in a manner consistent with the conventional utilization of antigens in vaccines. For example, the peptides of this invention may be combined with pharmaceutically 35 acceptable adjuvants conventionally employed in vaccines and administered in immunologically effec-

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tive amounts to protect patients for some time against HIV infection.

Both the compositions and vaccines of this invention may be administered to patients via conventional modes of administration. The frequency of administration will depend upon factors such as the particular composition or vaccine employed and the condition of the patient. The need for subsequent treatments with these compositions or boosters of these vaccines will depend upon the results of the initial treatment or vaccination.

In addition, the peptides of this invention and the antibodies raised to them may be employed in presently available methods and kits designed to detect the presence of HIV and antibodies to HIV in blood, organ or semen samples.

In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any manner.

#### EXAMPLE I

25      A. Preparation Of Peptides Involved In The Pathogenesis of HIV Infection

We synthesized peptides 1-6, 31, 64 and 78, corresponding to segments of the env gene of the HIV genome. These peptides are depicted in Figure 1, in which the amino acid numbering corresponds to that set forth for the env gene in L. Ratner et al., "Complete Nucleotide Sequence Of The AIDS Virus, HTLV-III", Nature, 313, pp. 277-84 (1985).

30      We synthesized the peptides using an improved version of the solid phase method described by R. B. Merrifield, "Solid Phase Peptide Synthesis. I. The Synthesis Of A Tetrapeptide", J. Am. Chem.

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Soc., 83, pp. 2149-54 (1963), using an Applied Bio-systems Model 430A peptide synth sizer and reagents and procedures as supplied by producer. In this improved method, we deblocked and cleaved the protected peptides from the resin with liquid HF containing 10% anisole, in a variation of the method described by S. Sakakibara et al., "Use Of Anhydrous Hydrogen Fluoride In Peptide Synthesis. I. Behavior of Various Protective Groups In Anhydrous Hydrogen Fluoride", Bull. Chem. Soc. Jap., 40, pp. 2164-68 (1967).

We first purified the peptides cleaved from the resin by partition chromatography on a Sephadex LH20 column using n-Butanol/water (6/100) as eluent. The eluate was further purified by semi-preparative high pressure reversed phase chromatography on an Altex Ultrasphere-ODS column, by elution with a 0.1% TFA acetonitril gradient. After we hydrolyzed the eluate with 6N HCl for 18 hours, we carried out amino acid analysis on a Beckman amino acid analyzer to confirm the amino acid sequences of the peptides produced.

B. Immunological Activity  
Of The Peptides Of This Invention

25 1. Coupling Of Peptides To Carrier Proteins

In one embodiment of this invention, peptides 1-6,\* 31, 64 and 78 were preferably coupled to one or more carrier proteins before use. Accordingly, we coupled each of those peptides prepared as described above to the carrier protein keyhole limpet haemocyanin (KLH, Sigma) by mixing 2 mg of peptide in

35 \* Due to an inadvertent error, the 873,621 application stated that peptides 2 and 3 were used in non-coupled form. Peptides 2 and 3 as employed in the examples of both the '621 application and the present application were used in coupled form.

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2 ml sodium phosphate buffer (0.1M, pH = 8) with 5 mg of KLH in 2 ml sodium phosphate buffer (0.1M, pH = 8). We then added 1 ml of a 0.25% glutaraldehyde solution to the mixture in several portions over a 5 period of 1 hour. We stirred the resulting mixture for another 6 hours and then dialyzed it against PBS overnight.

For use in vaccine compositions, the peptides of this invention may be coupled with tetanus 10 toxoid antigen, diphtheria toxoid antigen or another natural or synthetic carrier suitable for use in humans using conventional techniques. Alternatively, the peptides may be coupled with a suitable adjuvant 15 to enhance the immune response in the patient. The peptides may also be used in combination with any suitable synthetic low molecular weight carrier before use. Finally, an additional cysteine residue may be added to the C or N terminus of the peptide for coupling to a suitable carrier by disulfide linkage.

20 2. Inoculation Of Test Animals

We emulsified each of the KLH-coupled peptides 1-6, 31, 64 and 78 with Freund's complete adjuvant in a 1:1 ratio. Subsequently, we inoculated groups of 3 BALB/CJ mice (Jackson Laboratory, 25 Bar Harbor, Maine) by subcutaneous injection of 100 µg/250 µl of the emulsification into each mouse. On following days 14 and 35, each mouse received a booster injection of 100 µg/250 µl of the same coupled peptide emulsified 1:1 in Freund's incomplete adjuvant. 30 Tail bleeds were taken on days 21 and 42; with serum samples being stored at -20°C until the time of assay.

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3. Immunological Assaysi. ELISA With Antipeptide Sera Against env Peptide Coated Plates

- 5 In this assay, we determined that antiserum raised in an animal by each of peptides 1-6, 31, 64 and 78 of this invention binds to that peptide. Accordingly, the peptides of this invention are immunogenic and elicit a response in test animals.
- 10 To carry out the assay, we coated two of four 96-well microtiter plates (Munc Immuno I) with 50  $\mu$ l of a mixture of 50  $\mu$ g/ml uncoupled peptide in PBS (20mM phosphate, 150mM NaCl, pH = 7.2) and incubated the plates overnight at room temperature.
- 15 The third and fourth plates, which served as controls for, respectively, the first and second plates, were treated identically to those plates but were not pre-coated with peptide. We then inverted the plates to empty all wells and washed the plates 3 times
- 20 with PBS/0.05% Tween-20. The plates were blotted dry by gentle tapping over paper towels. After blotting the plates, we added 350  $\mu$ l of a 5% fetal calf serum/PBS ("FCS/PBS") solution to each well and incubated the plates for 1 hour at room temperature.
- 25 We then washed and blotted the plates as before.

We then assayed serum samples pooled from each group of 3 mice on the two pre-coated plates prepared as described above and on two control plates. In the first pre-coated plate, we assayed the antibody response to the immunogen peptide at an initial dilution of 1:10, followed by serial 2-fold dilutions in 5% FCS/PBS. In the second plate, an initial serum dilution of 1:20 was followed by serial 3-fold dilutions in 5% FCS/PBS.

35 After a 2 hour incubation period, we washed the plates and blotted them dry as described above. We then added 50  $\mu$ l of 1:2000 dilution of goat anti-

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mouse-IgG horseradish peroxidase ("HRP") (A.P., heavy and light chain specific, Cappel Laboratories) in 5% FCS/PBS to each well and incubated the plates at room temperature for 1 hour. We then washed the 5 plates with PBS/0.05% Tween-20. We added 42 mM of 3, 3', 5, 5'-tetramethylbenzidine in dimethylsulfoxide ("TMB/DMSO"), 7.35  $\mu$ l of 30% hydrogen peroxide ("H<sub>2</sub>O<sub>2</sub>") to 50 ml of 0.1M sodium acetate-citric acid buffer (pH = 4.92). Subsequently, we added 50  $\mu$ l of 10 this solution to the wells using a 12 channel multiple pipet. We stopped the enzyme reactions with 50  $\mu$ l of 2M H<sub>2</sub>SO<sub>4</sub> when the less dilute samples reached an absorbance of 0.2 O.D. at 650 nm. We then analyzed 15 the plates spectrophotometrically at 410 nm using a microplate reader (Dynatech MR600) and observed that antiserum against each of peptides 1-6, 31, 64 and 78 binds to that peptide.

ii. ELISA With Antipeptide Sera Against Virus-Coated Plates

20 In this assay, we demonstrated that antisera raised against the peptides of this invention binds to HIV virus-coated plates.

25 We added 100  $\mu$ l of carbonate buffer (pH = 9.6) containing 5% bovine serum albumin to each well of 96 well microtiter plates coated with authentic HIV virus (a gift of Dr. Robert Gallo) and incubated the plates at room temperature.\* Virus-coated micro-

30 \* Due to an inadvertent error, the 873,621 application referred to the preparation of HIV virus-coated plates. The virus-coated plates as employed in the examples of both the '621 application and the present application were a gift of Dr. Robert Gallo. It is our understanding that the plates used were in fact 35 made, or at least could have been made, by coating 96 well microtiter plates (Nunc Immuno I) with 100  $\mu$ l of a mixture of 5  $\mu$ g/ml authentic HIV virus in carbonate buffer (pH = 9.6), incubating the plates overnight at 4°C, inverting the plates to empty all 40 wells, washing the plates 3 times with deionized water and then blotting them.

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titer plates are also available from Electr nucleonics, Fairfield, New Jersey. Subsequently, we rinsed the plates 3 times with deionized water.

After blotting the plates, we added 100  $\mu$ l  
5 of saline-PO<sub>4</sub>(PBS) containing 20% normal goat serum to each well. We next added 5  $\mu$ l of human test serum or control serum to each well and incubated the plates overnight at 4°C, or for 2 hours at room temperature. We then washed the plates 3 times with PBS containing  
10 0.05% Tween-20 and blotted them.

We next added 100  $\mu$ l of a 1:4000 dilution of 1% normal goat serum and goat anti-human-IgG HRP (heavy and light chain specific) in 0.05% PBS-Tween 20 to each well and incubated the plates for 1 hour  
15 at room temperature. We had titrated the anti-human-IgG HRP before use to assure a proper final concentration of indicator antibody. At the end of the hour incubation period, we rinsed the plates 2 times with 0.05% PBS-Tween-20 and once with plain PBS. We then  
20 added 100  $\mu$ l of a solution of 0.005% H<sub>2</sub>O<sub>2</sub> and 0.05% orthophenylenediamine ("OPD")\* in Sorenson's phosphate citrate buffer (pH = 5) and allowed reaction for 20 minutes at room temperature in the dark.

We stopped the reaction by adding 50  $\mu$ l of  
25 4N H<sub>2</sub>SO<sub>4</sub> to each well. The plates were read by visual inspection or using a microplate reader at 490 nm.

Each plate had a series of "blank" control wells containing no human serum or anti-human IgG-HRP conjugate and to which one of the following had been  
30 added:

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\* This is a potential carcinogen which should be detoxified before disposal using a solution of:

35 50 g K<sub>2</sub>CrO<sub>7</sub>  
25 ml 10M H<sub>2</sub>SO<sub>4</sub>  
145 ml H<sub>2</sub>O

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- 5                   -- saline-PO<sub>4</sub> (PBS) containing 20%  
                  normal goat serum  
                  -- PBS-Tween-20 (0.05%)  
                  -- Sorenson's phosphate-citrate buffer  
                  (pH = 5) containing 0.005% H<sub>2</sub>O<sub>2</sub> and  
                  0.05% OPD.

In addition, each plate had a series of "background" control wells containing no human serum and to which one of the following had been added:

- 10                  -- saline-PO<sub>4</sub> (PBS) containing 20%  
                  normal goat serum  
                  -- PBS-Tween-20 (0.05%) containing 1%  
                  normal goat serum and goat -  
                  anti-human-IgG HRP at a dilution  
                  of 1:4000  
                  -- Sorenson's phosphate-citrate buffer  
                  (pH = 5) containing 0.005% H<sub>2</sub>O<sub>2</sub>  
                  and 0.05% OPD.

20                  Each test plate also had a negative and positive control serum. We tested antiserum raised against each of peptides 1-6, 31 and 64.

Analysis of the plates revealed that anti-serum raised against each of peptides 1, 2, 4 and 31 of this invention binds to the HIV virus.

25                  4. Virus Functional Assays

i. Syncytium Inhibition Assay

We assayed the peptides of this invention, as well as antisera raised to them, for their ability to inhibit syncytium formation in a variation of the 30 assay procedure set forth in C. D. Richardson and P. W. Choppin, "Oligopeptides That Specifically Inhibit Membrane Fusion By Paramyxoviruses: Studies On The Site Of Action", Virology, 131, pp. 518-32 (1983). In our assay, we added recombinant HIV env 35 protein, instead of live virus, to cultures of T-4 positive cells in the presence of one of the peptides of this invention, or antiserum raised thereto, and

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observed the degree of inhibition of syncytium formation and cell fusion in the cultures.

We demonstrated by this assay that the peptides of this invention, as well as antisera raised to them, inhibit virus-mediated events, such as virus adsorption to cells and syncytium formation. In the assay, peptides 1-6, 31 and 64 and antiserum raised against each of peptides 1-6, 31, 64 and 78 were tested. Only peptide 3 inhibited syncytium formation.\* Additionally, antiserum raised against each of peptides 1, 2, 4, 5, 31 and 78 inhibited syncytium formation.

Accordingly, this assay indicates the utility of the peptides of this invention and the antisera raised thereto as therapeutic agents. For example, administration of such peptides to an infected host may inhibit cell-to-cell transmission of the virus and virus-induced cell fusion sufficiently to prevent spread of the infection and ultimate destruction of the immune system. Alternatively, these peptides may be usefully administered in a priming dose which would permit a subsequently infected host to raise neutralizing antibodies effective against the virus.

25                   ii. Virus Neutralization Assay

a. HIV Neutralization Based On Lysis Of Cells

We assayed antisera raised to the peptides of this invention, to determine their ability to neutralize HIV virus based on lysis of cells. In this assay, we mixed HIV-sensitive cells with the

35                   \* Because peptide 64 did not inhibit syncytium formation in this assay and because peptide 64 is identical to peptide 3, the inhibitory activity previously reported for peptide 3 may have been attributable to impurities present with peptide 3 in the sample assayed.

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antisera, incubated them for several days and then observed the cells microscopically for lysis.

We observed that antisera to peptides 1, 2, 4, 5 and 31 of this invention neutralized HIV virus, preventing HIV infection and subsequent lysis of cells. Antiserum to each of peptides 6 and 78 did not display such activity and antisera to peptides 3 and 64 were not tested.

Such neutralizing activities indicate that the peptides of this invention and antisera thereto are useful in vaccines for preventing HIV infection. Alternatively, these peptides and antisera are useful in therapeutic compositions for inhibiting virus replication in an infected host.

15 C. Use Of The Peptides Of This Invention And Their Antibodies In Detecting HIV And Antibodies To HIV

Methods and diagnostic kits are presently available which are designed to detect the presence of HIV and antibodies to HIV. Peptides involved in the pathogenicity of HIV infection prepared by the processes of this invention and antibodies raised with them can also be employed in these methods and kits to detect the presence of HIV and antibodies to HIV. These peptides and their antibodies may be packaged in diagnostic kits which allow the rapid and convenient identification of AIDS carriers.

For example, the peptides of this invention or antibodies raised using them can be employed in the immunological diagnostic tests currently available for HIV antigen or antibody detection, e.g., radioimmunoassay or ELISA techniques.

In each assay, both the peptides of this invention and antibodies to these peptides, are used. The antibodies are produced by injecting laboratory animals with the peptides of this invention in a suitable solution, such as Freund's adjuvant, followed

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by bleeding the animal some six weeks later, removing the red blood cells by centrifugation, and using the resulting serum. Alternatively, monoclonal antibodies to the peptides of this invention may be produced

5 using standard hybridoma techniques.

In one type of radioimmunoassay, antibodies to an HIV peptide produced as above are attached to a solid phase, for example, the inside of a test tube. A sample of the patient's serum is added to

10 the tube, together with a known amount of a peptide of this invention, produced as above, and labelled with a radioactive isotope such as radioactive iodine.

15 Any HIV antigen in the patient's serum will compete with the labelled peptide for binding with the HIV antibodies. The excess liquid is removed, the test tube washed, and the amount of radioactivity measured. A positive result; i.e., that the patient's serum

contains HIV antigen, is indicated by a low radioactivity count left in the tube, as compared with a

20 control.

In one type of ELISA test, a microtiter plate is coated with a peptide prepared in accordance with this invention, and to this is added a sample of patient's serum. After a period of incubation

25 permitting interaction of any HIV antibody present in the serum with the HIV antigen, the plate is washed. A preparation of anti-human antibodies,

raised in a laboratory animal by injection of semi-purified human immunoglobulin, and then linked to an

30 enzyme, is added. Incubation allows an antibody-

antigen reaction to take place, and the plate is then rewashed. Thereafter, enzyme substrate is added to the microtiter plate and incubated for a period of time to allow the enzyme to react with the sub-

35 strate. The absorbance of the final preparation is then measured. A large change in absorbance indicates

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a positive result, i.e., that the patient's serum contains antibodies to HIV.

While we have hereinbefore presented a number of embodiments of this invention, it is 5 apparent that our basic construction can be altered to provide other embodiments which utilize the process of this invention. Therefore, it will be appreciated that the scope of this invention is to be defined by the claims appended hereto rather than the specific 10 embodiments which have been presented hereinbefore by way of example.

□

□

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We Claim:

1. A peptide involved in the pathogenesis of the HIV virus selected from the group consisting of peptides characterized by an amino acid sequence 5 derived substantially from the region between about amino acid 600 and amino acid 750 of the HIV env gene.

10 2. A peptide according to claim 1, selected from the group consisting of peptides characterized by a sequence of amino acids consisting substantially of amino acid sequences of the formulae:

PWNASWSNKSLEQIWNN, LLELDKWASLWNWF, EQIWNNMTWMEWD, LPIPRGFDRPECIEEGGERDRDR and D-retro forms thereof.

15 3. A peptide involved in the pathogenesis of the HIV virus characterized by a sequence of amino acids selected from the group consisting of peptides consisting substantially of amino acid sequences of the formulae:

20 RIKQIINMWQEVGKAMYAPPISGQI, VRKIEPLGVAPTKAKRPVQREKRA, NSSSGRMIMERGEIKNCS, SVEINCTRPNNNTRKSI and D-retro forms thereof.

C5  
Y3  
=

25 4. A pharmaceutically acceptable composition for treating patients to protect them for some time against HIV infection which comprises an immunologically effective amount of at least one peptide according any one of claims 1 to 3.

30 5. A method for treating patients to protect them for some time against HIV infection comprising the step of treating said patients in a pharmaceutically acceptable manner with the composition according to claim 4.

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6. A pharmaceutically acceptable composition for treating HIV infection in patients which comprises at least one peptide according to any one of claims 1 to 3 in an amount effective to lessen  
5 the severity of the HIV infection.
7. A method for treating HIV infection in patients comprising the step of treating said patients in a pharmaceutically acceptable manner with the composition according to claim 6.
- 10 8. A method for detecting antibodies to HIV antigen in samples of blood, tissue or semen characterized by at least one peptide according to any one of claims 1 to 3.
- 15 9. A method for detecting HIV antigens in samples of blood, tissue or semen characterized by an antibody to at least one peptide according to any one of claims 1 to 3.
- 20 10. An HIV diagnostic kit characterized by at least one peptide according to any one of claims 1 to 3 or at least one antibody raised against one or more of those peptides.

FIGURE 1AA 600-  
750 of  
HIV env

: GIWCCSGKLICTTEAVPMAS  
 WSNKSLEQIWMMMTWMEWDR  
 EINMYTSЛИHSLIKESQMQ  
 EIQDQELLELDKWAISLWMMF  
 NITDWLWYIKLFINIVCGLV  
 GLRIVPAVLSVVNRVQGYS  
 PLSFQTHLPIPRGPDPEGI  
 KKEGGKHDID

Peptide 1  
(AA 616-632  
of HIV env)

PMKIASWSNKSLEQIWMM

Peptide 2  
(AA 667-680  
of HIV env)

LLELDKWAISLWMMF

Peptide 3:  
(AA 627-639  
of HIV env)

EQIWMEMTRWMEWQ

Peptide 4  
(AA 728-751  
of HIV env)

LPIPRGPDRPEGIKKEGGKHDIDR

Peptide 5  
(AA 426-450  
of HIV env)

RIKQIINMWQEVGKAMIDAPPISQI

Peptide 6  
(AA 496-519  
of HIV env)

VKIEPLGVAPTKAKRRVVQREKRA

Peptide 31  
(AA 148-165  
of HIV env)

NSSSGRMIMERGEIKMCS

Peptide 64  
(AA 627-639  
of HIV env)

EQIWMEMTRWMEWQ

Peptide 78  
(AA 298-314  
of HIV env)

SVEINCTRPNNMTRKSI

SUBSTITUTE SHEET

201-202

\* Ext. end  
ext. amino  
acid  
amideGP H<sub>1</sub> N<sub>1</sub>  
valine  
sulf

C4

C5

V3

left out  
their  
last A  
raised  
ab  
against  
that  
region

Bond  
to virus  
if not  
cells  
WB  
V20  
DN neutrally

## INTERNATIONAL SEARCH REP RT

International Application No PCT/US87/01294

**L. CLASSIFICATION OF SUBJECT MATTER** (if several classification symbols apply, indicate all):  
 According to International Patent Classification (IPC) or to both National Classification and IPC  
 IPC (4) C07K 7/08, 7/10; A61K 39/12, 39/42; GOIN 33/53  
 US. CL. 530/324, 325, 326, 327; 424/88, 89; 435/7

**M. FIELDS SEARCHED**

Minimum Documentation Searched:

Classification System	Classification Symbols
US	530/324, 325, 326, 327; 424/88, 89; 435/7

Documentation Searched other than Minimum Documentation  
to the Extent that such Documents are Included in the Fields Searched:**CHEMICAL ABSTRACT AND BIOLOGICAL ABSTRACT ONLINE COMPUTER SEARCH****M. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category	Description of Document, <sup>14</sup> with indication, where appropriate, of the relevant passages	Relevant to Claim No. <sup>15</sup>
X, P	US, A, 4,629,783 (COSAND) 16 DECEMBER 1986, SEE CLAIM 6.	1, 4-10
X	SCIENCE, (WASHINGTON, D. C., USA), VOLUME 231 ISSUED MARCH 1986, (KENNEDY ET AL), "ANTI- SERUM TO A SYNTHETIC PEPTIDE RECOGNIZES THE HTLV-III ENVELOPE GLYCOPROTEIN", PAGES 1556-1559. SEE PAGE 1556 IN PARTICULAR.	1-2, 4-10
A	CELL, VOLUME 45, ISSUED 06 JUNE 1986, (STARCICH ET AL), "IDENTIFICATION AND CHARACTERIZATION OF CONSERVED AND VARIABLE REGIONS IN THE ENVELOPE GENE OF HTLV-III/LAV, THE RETROVIRUS OF AIDS, PAGES 637-648.	1-10

**\* Special categories of cited documents:**

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\*G\* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

\*H\* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

\*I\* document member of the same patent family

**IV. CERTIFICATION**

Date of the Actual Completion of the International Search:

15 JULY 1987

International Searching Authority:

ISA/US

Form PCT/ISA/210 (second sheet) (May 1986)

Date of Mailing of this International Search Report:

04 AUG 1987

Signature of Authorized Officer:

Christina Chan

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